I hereby certify that this correspondence is being deposited with the United States Postal Services on the date set forth below as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.

Date of Signature and Deposit: May 7, 1999 Joan C. Baker
Attorney of Record

#13 Declaration w/affidavit Mail 3/12/02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Kelly J. Henrickson, et al.
Serial No.:	08/691,045
Filed:	August 1, 1996
For:	VIRUS ASSAY METHOD
Group Art Unit:	1634
Examiner:	D. Rees

DECLARATION OF DR. KELLY HENRICKSON AND DR. JIANG FAN

Assistant Commissioner For Patents
Washington DC 20231

Dear Sir:

We, Dr. Kelly Henrickson and Dr. Jiang Fan, declare that:

1. We are the named inventors of the above-identified application.

2. We are both employed by the Medical College of Wisconsin. Dr. Henrickson's position is Associate Professor. He has studied detection of various viruses, emphasizing detection of human parainfluenza virus, for 12 years. Dr. Fan's position is Research Scientist, and he has studied detection of viruses for 20 years.

3. We disagree with the Examiner's characterization of the prior art cited in the November 10, 1998 Office Action because we disagree that one would read the combined

references as sugg sting that a PCR multiplex virus detection system, which we have described in the specification and claimed in the current pending claims, is disclosed by these references. The references do not teach a way of successfully detecting multiple viruses.

4. We state that we were unable to perform satisfactory multiplex detections of virus-containing samples without the unequal primer concentrations of the present invention. The example below at paragraph 5 illustrates the type of problem we have repeatedly encountered.

5. In experiments performed during the conception of the present invention, we found that an unequal primer concentration was crucial for successful multiplex detection.

6. We found that the unequal concentration of primer pairs ~~(with higher concentration for biotin-labeled primers)~~ significantly increased the multiplex RT-PCR-EHA sensitivity. For instance, we performed one experiment with HPIV-3 primers. One of the primers was labeled with biotin and the other was not labeled with biotin. The primers were in equal concentration, at 0.5 μM or 0.25 μM final concentration. With 0.05 ng HPIV-3 RNA, the RT-PCR-EHA O.D. readings were 0.200 or 0.257. However, with an unequal concentration primer pair, (biotin-labeled primer at 0.5 μM final concentration and non-labeled primer at 0.25 μM final

concentration) the O.D. reading increased to 0.717. That is a 2.79 - 3.56 fold increase. If we set a base-line cutoff at 0.300 to 0.400, the O.D. reading from equal concentration RT-PCR-EHA would appear to be negative, even if the sample was a positive one.

7. This distinction is an important one because a cut-off of 0.300 to 0.400 is a common base-line cutoff. This cutoff is used for most commercial PCR Assays (e.g. Roche's ~~HBV~~ HIV PCR).

8. We further state that we have reviewed the Wu, et al. reference and find that this reference is not drawn to the creation of double-stranded molecules but is "asymmetric PCR." An asymmetric PCR reaction will produce an abundance of single-stranded molecules and is not suitable for multiplex detection.

9. We have been carefully following the scientific literature concerning multiple detection of viruses for the past 10 years. To our knowledge, no other researchers have appreciated the advantages of our unequal primer concentration reaction composition. We point as an example to the attached Grondahl, et al., a recent example of a multiplex identification of numerous microorganisms. We note that Grondahl, et al. use equal concentrations of labeled and unlabeled primers. The results in Grondahl, et al. are inferior to ours because their reported sensitivity is 30-50% lower. For example, they missed 17% of RSV EIA

positive specimens. In a comparison test, our method detected all RSV EIA positive sp cimens plus 30% mor true positive sp cimens missed by EIA.

10. We declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Respectfully submitted,

5/5/99
Date

Kelly J Henrickson
Kelly Henrickson

5/5/99
Date

Jiang Fan